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First Record of *Acrobeloides nanus* (Cephalobidae: Rhabditida: Nematoda) from Korea

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ABSTRACT

Acrobeloides nanus (de Man, 1880) Anderson, 1968 belonging to the family Cephalobidae Filpijev, 1934 (Cephalobomorpha) is newly reported from South Korea. This species is distinguished from other *Acrobeloides* species by its low and blunt labial probolae, five lateral incisures with middle incisure extending to the tail tip, and bluntly rounded tail. In this study, details of morphological characters of *A. nanus* is described and illustrated based on optical and scanning electron microscopy. In addition, molecular sequence data of the D2−D3 region of 28S rDNA, 18S rDNA and mitochondria DNA *cox1* region from this species are provided as DNA barcode sequences.

Keywords: Cephalobidae, *Acrobeloides nanus*, SEM, molecular, new record

INTRODUCTION

Genus *Acrobeloides* (Cobb, 1924) Thorne, 1937 are bacterial feeding nematodes and are widely distribute in various terrestrial environments such as forests (Háněl, 1999), sand dunes (Yeates, 1967), and agricultural land (Pervez, 2011). Species in this group have been studied extensively (Thorne, 1937; Brezeski, 1962; Anderson, 1965, 1968; Andrássy, 1984; Siddiqi et al., 1992). To date, only one *Acrobeloides* species (unidentified at the species level) has been reported in Korea (Kim et al., 2012).

Following a survey of several plots of farmland, *A. nanus* (de Man, 1880) Anderson, 1968, were isolated from soil samples from potato farms. In this paper, we provide details of a morphological characters and morphometrics for this species from optical microscope and scanning electron microscope (SEM) images. In addition, molecular sequence information of the D2−D3 region of the 28S rDNA, 18S rDNA, and mitochondrial DNA *cox1* region from this species are provided as DNA barcode sequence data.

MATERIALS AND METHODS

Nematode isolation and culture

Nematode specimens were extracted from potato farm soil from Hapcheon-gun, Gyeongsangnam-do, South Korea (GPS coordinates: 35°27′37.4″N, 128°00′19.6″E), using sieving and the Baermann funnel method. One individual nematode was transferred to a soil agar plate (25 mg/mL autoclaved soil, 5 μg/mL cholesterol, and 1% agar) and cultured at room temperature (18−20°C).

Fixation and morphological observation

For fixation, the nematode specimen was transferred to 2 mL water in a 15 mL tube, to which was added 4 mL of 80°C TAF (2% triethanolamine and 7% formaldehyde). The fixed nematodes were processed to dehydrated glycerin using Seinhorst's (1959) method and mounted in pure glycerin on permanent HS-slides (Shirayama et al., 1993). Morphological characters of nematode specimens were observed under an optical microscope (BX-51; Olympus, Tokyo, Japan) equipped with differential interference contrast, and morpho-

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metric characters were measured using a CoolSnap Photometrics color CCD digital camera (MP5.0-RTV-R-CLR-10; Photometrics, Tucson, AZ, USA) and the programQCapture Pro 5 (QImaging, Surrey, Canada).

Scanning electron microscope (SEM)

For SEM imaging, nematode specimens were fixed using TAF and maintained for a minimum of 24 h at room temperature. They were then transferred to a 4% aqueous osmium tetroxide solution and kept at 4°C for 3 days for postfixation. Fixed nematode specimens were dehydrated through a 10%−100% pure ethanol series for 1 h. The samples were dried using a Hitachi HCP-2 critical point drier (Tokyo, Japan). Dried nematodes were mounted on copper/nickel tape and sputter coated with gold/palladium using an Eiko IB-3 ion coater (Tokyo, Japan). Morphological characters of the nematode specimens were observed with a Zeiss Ultra Plus SEM (Oberkochen, Germany) at 15 kV under high-vacuum conditions.

Molecular techniques and SEQUENCES analysis

Total genomic DNA from *A. nanus* was extracted using an Epicentre MasterPure DNA Purification Kit (Epicentre, Madison, WI, USA) following the manufacturer's protocol. For amplification of the D2−D3 regions of 28S rDNA, 18S rDNA, and mitochondrial DNA *cox1* fragments, polymerase chain reaction (PCR) was performed using universal primer sets (D2A [5′-ACAAGTACCGTGAGGGAAAGTTG-3′]/D3B [5′-TCGGAAGGAACCAGCTACTA-3′]; De Ley et al., 1999 for D2−D3 region of 28S and 328-F [5′-TACCTGGTTGATCCTGCCAG-3′]/329-R [5′-TAATGATCCTTCCGCAGGTT-3′]; Adl et al., 2014 for 18S) and a nematode-specific primer set (Cepha_CO1_F [5′-ATGATTTTTTTTATGGTGATGCC-3′]/Cepha_CO1_R [5′-ACTACAAAATATGTGTCATG-3′] for *cox1* region) that was designed based on conserved regions of nematode mitochondrial genes. PCR reactions were performed in a total volume of 50 μL including 2 μL template DNA, 10 pmol of each primer, 10× Ex Taq buffer, 0.2 mM dNTP mixture, and 1.25 U of Taq polymerase (TaKaRa Ex Taq). PCR amplification conditions were as follows: initial denaturing step at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min (extended to 2 min for the 328/329 primer) followed by a final extension at 72°C for 10 min. The amplified PCR products were purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. Big Dye Terminator Cycle-Sequencing (Applied Biosystems, Waltham, MA, USA) was used for sequencing the PCR-amplified fragments.

The obtained sequences of the D2−D3 region of 28S rDNA, 18S rDNA, and mtDNA *cox1* region from the specimens were aligned with sequences of other acrobeloids available from GenBank, using Clustar X with default options (Thompson et al., 1997). Both ends of the aligned datasets were trimmed before sequence analyses.

SYSTEMATIC ACCOUNTS

Order Rhabditida Chitwood, 1933
Suborder Tylenchina Thorne, 1949
Infraorder Cephalobomorpha De Ley and Blaxter, 2002
Family Cephalobidae Filipjev, 1934
Genus *Acrobeloides* (Cobb, 1924) Thorne, 1937

[1]*_Acrobeloides nanus_ (de Man, 1880) Anderson, 1968 (Table 1, Figs. 1, 2)

Cephalobus nanus de Man, 1880: 39.
Acrobeloides nanus: Anderson, 1968: 309, figs. 3−5.

Material examined. 18♀♀, Korea: Gyeongsangnam-do, Hapcheon-gun, Gahoe-myeon, 26 Mar 2015, extracted by sieving and the Baermann funnel method from potato farm soil. Two specimens (slide Nos. NIBRIV0000326012 and NIBRIV0000326013) are deposited at the National Institute of Biological Resources, Republic of Korea. Sixteen specimens (slide Nos. 01010503001−01010503016) are deposited in the Animal Phylogenomics Laboratory, Ewha Womans University, Republic of Korea.

Measurements. See Table 1.

Description. Female: Body cylindrical, length 335.3−442.3 μm, ventrally curved after fixation (Fig. 1A). Cuticle annulated; annuli 1.6−2.2 μm wide and 0.5−0.8 μm thick at midbody. Lateral incisures varying in number along body length: three incisures at procorpus region, branching off from deirid into five incisures, and three incisures at anterior anus; two incisures fading out around phasmid; middle incisure extending to near tail end (Figs. 1A, 2B, D). Head region continuous with neck. Lip region 6.6−7.9 μm wide, triradiate symmetry with 6+4 papillae. Cephalic probolae absent; three straight, conical-rounded labial probolae present. Amphid openings present, transversely opening, and oval shaped (Figs. 1B, 2A). Stoma cephaloboid with length about 1.6−2 times lip region diameter; bar-shaped cheilorhabdions, with dorsal denticle on metastom. Pharyngeal corpus fusiform with swollen metacorpus, 3−5.2 times isthmus length. Isthmus narrower than corpus, distinctly demarcated from

Korean name: [1]*난쟁이선충(신칭)

Table 1. Morphometrics of *Acrobeloides nanus*

	Acrobeloides nanus (♀, n=18)
L	408.6±29.0 (335.3–442.3)
Body width	21.3±2.1 (17.1–24.5)
Pharynx length	124.3±5.5 (111.6–128.8)
Tail length	23.4±1.4 (20.5–25.4)
Anal region body width	12.5±1.0 (10.5–14.0)
a	19.2±1.1 (17.1–21.5)
b	3.3±0.1 (3.0–3.5)
c	17.5±0.8 (15.8–19.0)
c′	1.9±0.1 (1.8–2.0)
Lip region width	7.4±0.3 (6.6–7.9)
Stoma	13.2±1.2 (11.7–15.7)
Corpus	70.4±4.4 (62.6–78.5)
Isthmus	19.1±3.1 (13.8–23.5)
Bulbus	20.3±1.8 (15.6–23.1)
Stoma/lip region width	1.8±0.1 (1.64–2.0)
Corpus:isthmus	3.8±0.8 (3.0–5.2)
Nerve ring	82.4±3.7 (74.7–87.5)
Excretory pore	86.0±3.4 (79.0–90.1)
Deirid	94.6±3.8 (86.6–99.7)
Nerve ring (% pharynx)	66.4±1.9 (61.9–69.5)
Excretory pore (% pharynx)	69.3±2.1 (64.7–73.7)
Deirid (% pharynx)	75.9±2.4 (72.8–81.1)
Rnr[a]	41.6±2.8 (38–51)
Rep[b]	43.8±3.0 (41–54)
Rdei[c]	49.1±3.0 (47–59)
Vulva from anterior end	266.0±15.6 (221.3–285.0)
V (%)	65.2±1.6 (63.6–70.2)
Reproductive tract length	111.5±18.9 (71.4–149.8)
G (%)	27.1±3.1 (20.5–33.9)
Vagina	6.2±0.9 (4.2–7.5)
Uterus	34.5±3.4 (29.5–39.8)
Uterus/body width	1.6±0.2 (1.4–1.8)
Spermatheca	10.5±2.6 (7.4–19.6)
Rectum	15.2±1.4 (13.0–17.7)
Rectum/anal width	1.2±0.1 (1.0–1.5)
Phasmid	7.8±1.4 (5.0–10.0)
Phasmid (% tail)	33.3±5.8 (23.7–43.3)
Tail annuli[d]	10.7±1.3 (8–13)
Cuticle thickness	0.6±0.1 (0.5–0.8)
Annuli width	1.9±0.2 (1.6–2.2)

All measurements are in μm and in the form mean±SD (range).
[a]Number of annules from the anterior end to the nerve ring.
[b]Number of annules from the anterior end to the excretory pore.
[c]Number of annules from the anterior end to the deirid.
[d]Number of annules from the anus to the tail end.

metacorpus. Basal bulb oval-shaped, with well-developed valves at middle part; cardia conoid, surrounded by intestinal tissue. Nerve ring located posterior of corpus to anterior isthmus region, 38–51 annuli from head, at 61.9%–69.5% of pharynx length. Excretory pore at posterior corpus to isthmus level, 41–54 annuli from anterior end, at 64.7%–73.7% of pharynx length. Deirid in lateral field at isthmus level, 47–59 annuli from anterior end, at 72.8%–81.1% of total neck length (Fig. 1B). Female reproductive system monodelpic-prodelpic. Vulva not protruding, vagina one-third of body width, postvulval sac rudimentary. Uterus tubular, 1.4–1.8 times body diameter. Spermatheca short (7.4–19.6 μm). Oviduct 1.5 times body width long, Ovary straight to posterior, sometimes with double flexure (n=2) (Figs. 1C, 2C). Rectum length 1.0–1.5 times anal body width. Tail conoid with rounded terminus, with 8–13 annules. Phasmids at 23.7%–43.3% of tail length (Figs. 1D, 2D).

Male: Unknown.

Distribution. Australia (Bird et al., 1993), Brazil (Rashid et al., 1984), Canada (Anderson, 1968), Falkland Islands (Boström, 1996), South Georgia Island (Boström, 1996), Krakatau (Rashid et al., 1988), Malaysia (Boström, 1993), Korea (present study), Sweden (Boström and Gydemo, 1983).

Habitat. Soil sample in the potato farm.

Remarks. Anderson (1968) proposed that in *A. nanus*, there are intraspecific variations in some morphologies depending on environmental conditions, such as measurements, shape of labial probolae (low-rounded, knobbed, conoid, and apiculate) and tail (hemispherical, clavate, conoid-truncated, and conoid-rounded) and position of phasmid, nerve ring, excretory pore and deirid. The aforementioned morphological variability among *A. nanus* populations has also been reported from many geographic areas: Australia (Bird et al., 1993), Brazil (Rashid et al., 1984), Canada (Anderson, 1968), Falkland Islands (Boström, 1996), South Georgia Island (Boström, 1996), Krakatau (Rashid et al., 1988), Malaysia (Boström, 1993), and Sweden (Boström and Gydemo, 1983). The morphological characters of the specimens observed from the present study are within the range of intraspecific variation reported from other localities in earlier studies (Table 2).

Identifying characteristics that distinguish *A. nanus* from *A. buetschlii* (de Man, 1884) Steiner and Buhrer, 1933 have long been debated. Anderson (1968) and Zell (1987) distinguished between *A. nanus* and *A. buetschlii* by the number of lateral incisures (five vs. three) and absence/presence of a postvulvar uterine branch (PUB). However, Rashid et al. (1984) reported three lateral incisures in *A. nanus* from a Brazil population. In addition, Bird et al. (1993), Boström (1993, 1996), and Rashid et al. (1984) refuted morphological differences (number of lateral incisures and absence or presence of PUB) between *A. nanus* and *A. buetschlii*.

Molecular sequence information. Molecular sequences deposited on GenBank: D2–D3 region in 28S rDNA (GenBank accession No. KX669640); 18S rDNA (GenBank accession No. KX669638); *cox1* of mtDNA (GenBank accession No. KX669639).

Molecular information. The sequences of the D2–D3 region of 28S rDNA, 18S rDNA, and the partial *cox1* gene of mitochondrial DNA were obtained from *A. nanus* (GenBank accession Nos. KX669640 [D2–D3 region of 28S rDNA], KX669638 [18S rDNA], and KX669639 [partial *cox1* gene

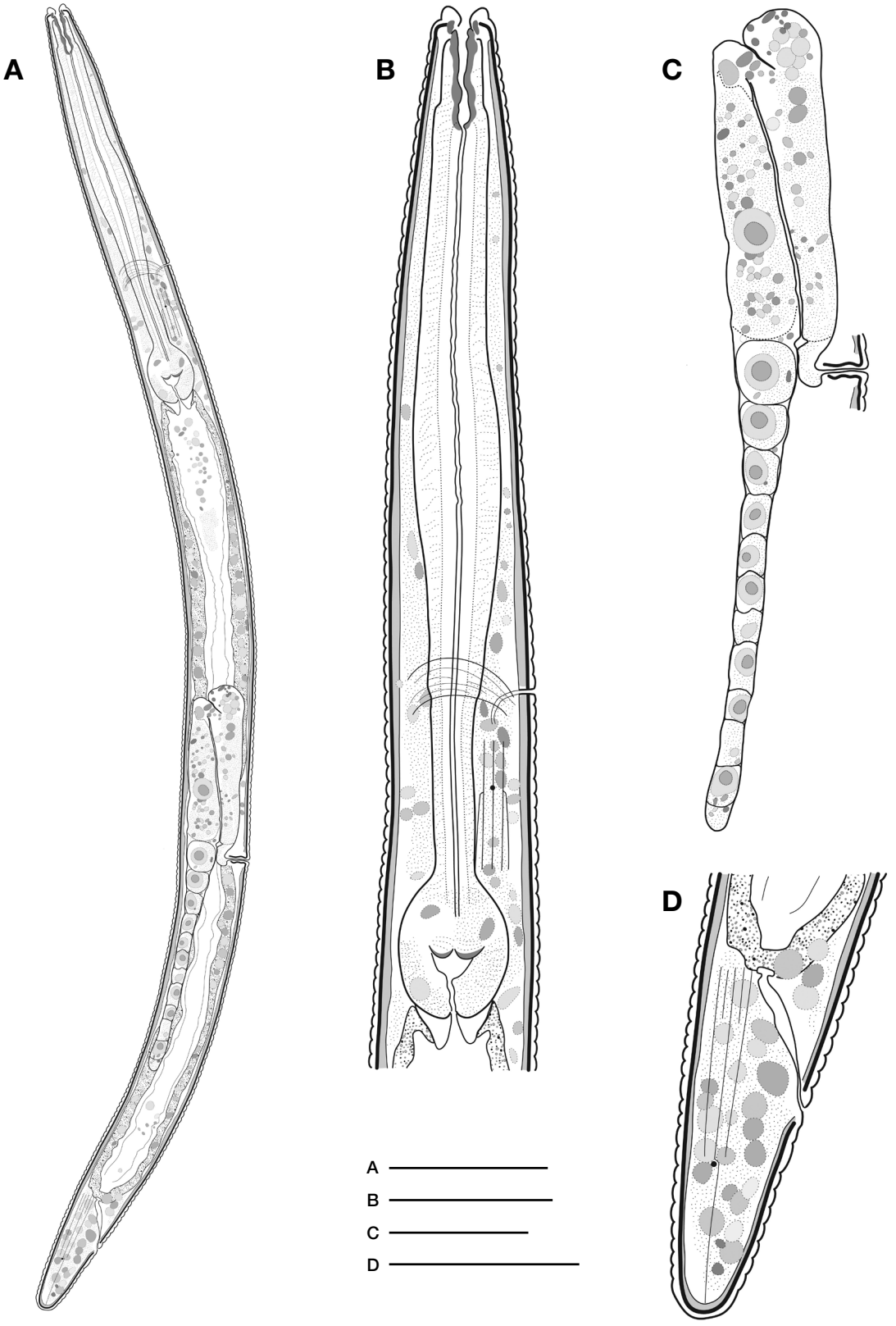

Fig. 1. *Acrobeloides nanus* (de Man, 1880) Anderson, 1968. A, Entire female; B, Neck region; C, Female reproductive system; D, Female posterior region. Scale bars: A = 50 µm, B–D = 20 µm.

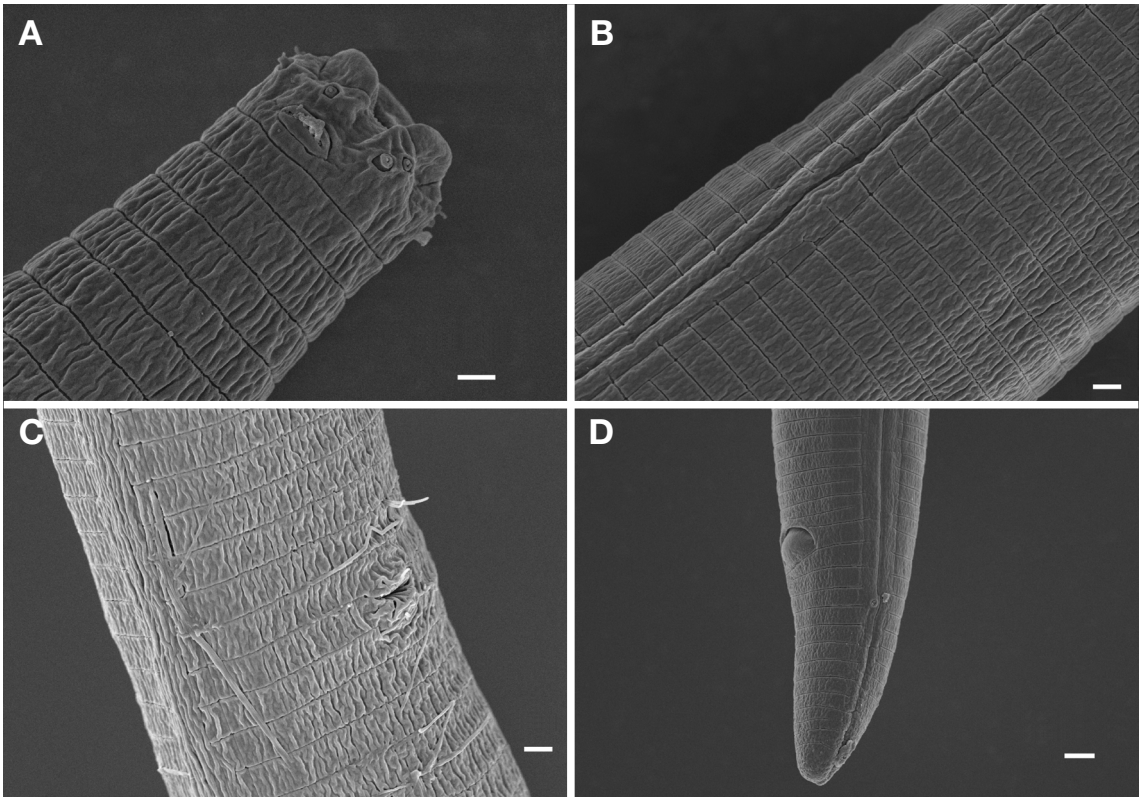

Fig. 2. *Acrobeloides nanus* (de Man, 1880) Anderson, 1968 (scanning electron microscopy). A, Head region; B, Lateral field at deirid region; C, Vulva; D, Tail region. Scale bars: A–C = 1 μm, D = 2 μm.

of mtDNA]) and compared with other acrobeloids available on GenBank. The *cox1* sequences from other *Acrobeloides* species are not yet available on GenBank. We provide *cox1* sequence data from *A. nanus* in this study for use in molecular barcoding.

The sequence of the D2–D3 region of 28S rDNA from *A. nanus* in this study is the same as *A. thornei* (DQ903083) and differs by one to five nucleotides from *A. buetschlii* (DQ903081; 3 bp), *A. ellesmerensis* (DQ145624; 3 bp), *A. uberrinus* (DQ903087; 3 bp), and *A. nanus* specimens from Jädras in Sweden (DQ903076; 1 bp), Cologne in Germany (EF417139; 1 bp), Sollentuna in Sweden (DQ903075; 2 bp), and Bourges in France (DQ903103; 5 bp). The sequence of the 18S rDNA from *A. nanus* in this study is the same as *A. buetschlii* (JQ957905), and differs by one or three nucleotides from *A. nanus* from an unknown location (DQ102707; 1 bp), *A. apiculatus* (AY284673; 1 bp) and *A. thornei* (JQ 957903; 3 bp). However, intraspecific variation of D2–D3 region sequences among some *A. nanus* populations is higher than interspecific variation among several *Acrobeloides* species. For example, the D2–D3 region sequences of *A. ellesmerensis* (DQ145624), *A. uberrinus* (DQ903078), and *A. buetschlii* (DQ903081) are identical, but D2–D3 sequences between different isolates of *A. nanus* (DQ903075 [Sweden], DQ903103 [France]) differ by 7 nucleotides. Also, the 18S rDNA sequences of *A. nanus* from an unknown location (DQ 102707) is distinguished by two base pairs from *A. apiculatus* (AY284673) and *A. nanus* in this study (KX669638). As described, molecular sequence data of *A. nanus* was the same or very similar to some other acrobeloids; however, its morphology clearly distinguishes *A. nanus* from *A. thornei* (with two lateral incisures, setose labial probolae and pointed tail), *A. buetschlii* (with three lateral incisures), *A. ellesmerensis* (with four lateral incisures with three extending to tail end, and setose labial probolae), *A. uberrinus* (with two to three incisures extending to tail end, and setose labial probolae), and *A. apiculatus* (with a pointed tail). In addition, earlier studies have reported that the D2–D3 region of 28S rDNA and the 18S sequence did not show clear resolution in their relationships among some species within Cephalobidae (Holterman et al., 2006; Nadler et al., 2006; Smythe and Nadler, 2006; Sonnenberg et al., 2007; Rybarczyk-Mydłowska et al., 2012). Therefore, the D2–D3 region of 28S rDNA and 18S rDNA should be used with great caution as molecular markers for species level identification of *Acrobeloides* species.

Table 2. Morphometrics and morphological variability among *Acrobeloides nanus* (de Man, 1880) Anderson, 1968 populations

	South Korea	Australia	Brazil	Canada	South Georgia	East Falkland Island	Krakatau	Malaysia	Sweden
L (μm)	335–442	359–452	300–540	321–497	345–459	258–458	280–400	328–407	306-403
a	17–22	14–18	17–27	15–24	17–22	13–19	16–24	14–20	15-24
b	3.0–3.5	3.3–3.9	3.2–4.5	2.8–4.1	3.2–3.6	3.2–4.3	3.0–3.7	3.3–4.0	2.9-4.0
c	16–19	22–28	13–21	14–23	14–19	17–26	13–20	12–16	13-19
V	64–70	65–69	59–72	62–70	61–66	65–68	64–68	63–68	60-69
Nerve ring	Posterior corpus-anterior isthmus	Anterior isthmus-middle isthmus	Metacorpus-posterior isthmus	Metacorpus-posterior isthmus	Corpus-isthmus junction-anterior isthmus	Anterior isthmus-middle isthmus	–	Anterior isthmus-posterior isthmus	–
Excretory pore	Posterior corpus-posterior isthmus	Anterior isthmus-posterior isthmus	Metacorpus-posterior isthmus	Metacorpus-posterior isthmus	Corpus-isthmus junction-middle isthmus	Corpus-isthmus junction-middle isthmus	–	–	–
Deirid	Middle isthmus-basal bulb	Posterior isthmus	Anterior isthmus-basal bulb	Anterior isthmus-basal bulb	Middle isthmus-posterior isthmus	Basal bulb	–	Posterior isthmus-basal bulb	–
Phasmid (% tail)	Anterior tail (24–43)	Middle tail (33–52)	Anterior tail-posterior tail	Anterior tail-posterior tail	Anterior tail (28–38)	Anterior tail (23–35)	–	Anterior tail (18–40)	–
Labial probolae	Conoid-rounded	Blunt, low, ridge	Apiculate, conoid, knobbed, low-rounded	Apiculate, conoid, knobbed, low-rounded	Conoid, knobbed	Conoid, knobbed, low-rounded, squared	Knobbed	Conoid, knobbed	Conoid, knobbed, square
Tail	Conoid-rounded	Bluntly rounded	Clavate, conoid-rounded, conoid-truncated, hemispherical	Clavate, conoid-rounded, conoid-truncated, hemispherical	Conoid-rounded	Conoid-cylindrical with broadly rounded	Conoid-rounded	Conoid, irregular, mucronate, pointed, rounded	Conoid-blunted, conoid-rounded, rounded

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